

CLAIMS

1. Process for the preparation of 1,3-propanediol from a carbon-containing substance, said process comprising at least one step which
5 consists of growing a recombinant micro-organism in which has been introduced at least one nucleic acid coding for the two subunits of a glycerol dehydratase the catalytic activity of which is independent of the presence of coenzyme B12 or one of its precursors.

2. Process according to Claim 1, characterized in that the glycerol
10 dehydratase the catalytic activity of which is independent of the presence of coenzyme B12 or one of its precursors is derived from *Clostridium butyricum*.

3. Process according to one of Claims 1 or 2, characterized in that
15 the glycerol dehydratase, the catalytic activity of which is independent of the presence of co-enzyme B12 or one of its precursors, is a dimeric protein composed of a first polypeptide having at least 50 % amino acid identity with the polypeptide of sequence SEQ ID No.6 and a second polypeptide having at least 50 % amino acid identity with the polypeptide of sequence
SEQ ID No.7.

4. Process according to one of Claims 1 to 3, characterized in that
20 the recombinant micro-organism comprises in addition a nucleic acid coding for a 1,3-propanediol dehydrogenase and preferably a 1,3-propanediol dehydrogenase of *Clostridium butyricum* VPI 1718.

5. Process according to Claim 4, characterized in that the 1,3-
25 propanediol dehydrogenase is a polypeptide having at least 90 % amino acid identity with the polypeptide of sequence SEQ ID No. 8.

6. Process according to one of Claims 1 to 5, characterized in that
the culture step of the recombinant micro-organism is carried out in the
absence of coenzyme B12 or one of its precursors.

7. Process according to one of Claims 1 to 6, characterized in that
30 the carbon-containing substance is selected from the carbohydrates and the polyols.

8. Process according to Claim 7, characterized in that the
carbohydrate is glucose and the polyol is glycerol.

9. Process according to one of Claims 1 to 8, characterized in that the micro-organism is selected from the micro-organisms not producing coenzyme B12 or one of its precursors naturally.

5 10. Process according to Claim 9, characterized in that it is a bacterium, a yeast or a fungus.

11. Process according to Claim 10, characterized in that it is a bacterium belonging to the *Clostridium*, *Escherichia*, *Bacillus*, *Lactobacillus* or *Lactococcus* genus.

10 12. Process according to Claim 10, characterized in that it is the yeast *Saccharomyces cerevisiae*.

13. Process according to one of Claims 1 to 12, characterized in that the recombinant micro-organism also comprises nucleic acids coding for a glycerol-3-phosphate dehydrogenase and a glycerol-3-phosphatase.

15 14. Nucleic acid comprising all or part of a polynucleotide coding for at least one subunit of a glycerol dehydratase, the catalytic activity of which is independent of the presence of coenzyme B12 or one of its precursors.

20 15. Nucleic acid according to Claim 14, characterized in that it comprises all or part of a polynucleotide having at least 50 % nucleotide identity with the polynucleotide of sequence SEQ ID No. 1 or SEQ ID No.2 or a polynucleotide with a complementary sequence.

25 16. Nucleic acid according to Claim 15, characterized in that it comprises a first polynucleotide having at least 50 % nucleotide identity with the polynucleotide of sequence SEQ ID No.1 and a second polynucleotide having at least 50 % nucleotide identity with the polynucleotide of sequence SEQ ID No.2.

30 17. Nucleic acid according to Claim 16, characterized in that it comprises in addition a sequence with a transcription promoter function, functional in the host cell in which the expression of the polynucleotide is desired.

18. Nucleic acid according to Claim 17, characterized in that the promoter sequence is the sequence SEQ ID No.3 or a sequence having at least 80 % nucleotide identity with this latter.

19. Nucleic acid with bacterial promoter function comprising a polynucleotide having at least 80 % nucleotide identity with the sequence SEQ ID No.3, or a polynucleotide with a complementary sequence.

5 20. Nucleic acid comprising all or part of a polynucleotide coding for a 1,3-propanediol dehydrogenase having at least 90 % nucleotide identity with the polynucleotide of sequence SEQ ID No.4, or a polynucleotide with a complementary sequence.

21. Nucleic acid comprising from the 5' end to the 3' end a first nucleic acid according to Claim 16 and a second nucleic acid according to
10 Claim 20.

22. Nucleic acid according to Claim 21, characterized in that it is a polynucleotide having at least 50 % nucleotide identity with the nucleotide sequence SEQ ID No.5.

23. Nucleic acid according to Claim 21, characterized in that it
15 comprises in addition a third nucleic acid coding for a glycerol-3-phosphate dehydrogenase and a fourth nucleic acid coding for a glycerol-3-phosphatase.

24. Recombinant cloning and/or expression vector comprising a nucleic acid according to one of Claims 16 to 23.

20 25. Recombinant vector according to Claim 24, characterized in that it is the plasmid pSPD5 contained in the *Escherichia coli* strain filed at the National Collection of Cultures of Micro-organisms (NCCM) on 24 June 1999 under the access No.I-2243.

26. Recombinant host cell comprising a nucleic acid according to
25 one of Claims 16 to 23 or a recombinant vector according to one of the Claims 24 to 25.

27. Recombinant host cell according to Claim 26, characterized in that it is the *Escherichia coli* strain filed at the National Collection of Cultures of Micro-organisms (NCCM) on 24 June 1999 under the access
30 No.I-2243.

28. Polypeptide encoded in a nucleic acid according to one of Claims 14 to 16, comprising all or part of an amino acid sequence having at least 50 % amino acid identity with the sequence SEQ ID No.6 or SEQ ID No.7.

29. Dimeric protein composed of a first polypeptide having at least 50 % amino acid identity with the polypeptide of sequence SEQ ID No.6 and of a second polypeptide having at least 50 % amino acid identity with the polypeptide of sequence SEQ ID No.7.

- 5 30. Polypeptide encoded in a nucleic acid according to Claim 20, comprising all or part of an amino acid sequence having at least 90 % amino acid identity with the sequence SEQ ID No.8.

31. Process for the production of a polypeptide according to one of Claims 28 to 30, characterized in that it comprises the following steps :

- 10 a) preparation of a recombinant expression vector according to one of Claims 24 and 25 ;
 b) introduction of the recombinant expression vector of step a) into a suitable host cell ;
 c) culture of the recombinant host cell of step b) into a suitable culture
15 medium ;
 d) recovery of the recombinant polypeptide produced from the culture supernatant or from the cell lysate ;
 e) if necessary, purification of the polypeptide recovered.

- 20 32. Antibody directed against a polypeptide according to one of Claims 28 to 30.